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Comparison of Fractional Inhibitory Concentration Index with Response Surface Modeling for Characterization of In Vitro Interaction of Antifungals against Itraconazole-Susceptible and -Resistant *Aspergillus fumigatus* Isolates

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Although the fractional inhibitory concentration (FIC) index is most frequently used to define or to describe drug interactions, it has some important disadvantages when used for drugs against filamentous fungi. This includes observer bias in the determination of the MIC and no agreement on the endpoints (MIC-0, MIC-1, or MIC-2 [≥95, ≥75, and ≥50% growth inhibition, respectively]) when studying drug combinations. Furthermore, statistical analysis and comparisons are troublesome. The use of a spectrophotometric method to determine the effect of drug combinations yields quantitative data and permits the use of model fits to the whole response surface. We applied the response surface model described by Greco et al. (W. R. Greco, G. Bravo, and J. C. Parsons, Pharmacol. Rev. 47:331–385, 1995) to determine the interaction coefficient alpha (ICα) using a program developed for that purpose and compared the results with FIC indices. The susceptibilities of amphotericin B (AM), itraconazole (IT), and terbinafine (TB) were tested either alone or in combination against 10 IT-susceptible (IT-S) and 5 IT-resistant (IT-R) clinical strains of *Aspergillus fumigatus* using a modified checkerboard microdilution method that employs the dye MTT [3-(4,5-dimethyl-2-thiazyl)2,5-diphenyl-2H-tetrazolium bromide]. Growth in each well was determined by a spectrophotometer. FIC indices were determined and ICα values were estimated for each organism strain combination, and the latter included error estimates. Depending on the MIC endpoint used, the FIC index ranged from 1.016 to 2.077 for AM-IT, from 0.544 to 1.767 for AM-TB, and from 0.656 to 0.740 for IT-TB for the IT-S strains. For the IT-R strains the FIC index ranged from 0.308 to 1.767 for AM-IT, from 0.512 to 1.646 for AM-TB, and from 0.403 to 0.497 for IT-TB. The results indicate that the degree of interaction is not only determined by the agents themselves but also by the choice of the endpoint. Estimates of the ICα values showed more consistent results. Although the absolute FIC indices were difficult to interpret, there was a good correlation with the results obtained using the ICα values. The combination of AM with either IT or TB was antagonistic in vitro, whereas the combination of IT and TB was synergistic in vitro for both IT-S and IT-R strains. The use of response surface modeling to determine the interaction of drugs against filamentous fungi is promising, and more consistent results are obtained by this method than by using FIC indices.

Invasive aspergillosis remains a serious opportunistic fungal infection, particularly in patients with a reduced immune defense, such as those with hematological malignancy or transplant recipients (9, 17). Mortality of invasive infections due to *Aspergillus* spp. is still high (9). The main reasons for this are the difficulty in diagnosing these infections, refractory underlying diseases and the limited efficacy of antifungal agents. Resistance of *Aspergillus fumigatus* against antifungal azoles and of *Aspergillus terreus* against amphotericin B (AM) has been described (14). Furthermore, the dose of conventional agents such as AM is restricted due to unwanted side effects.

Although AM remains the standard therapy for the treatment of invasive aspergillosis (14), the efficacy of this drug is limited. Several alternative antifungal agents are under investigation, such as the intravenous formulation of itraconazole (IT), voriconazole, and caspofungin. Although voriconazole was recently shown to be superior to AM for first-line treatment of invasive aspergillosis, the clinical efficacy was below 60% (R. Herbrecht et al., Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. 680, 2001).

An alternative approach to improve the survival of patients with invasive aspergillosis could be to combine antifungal agents. Combination therapy has been shown to be beneficial for several difficult-to-treat infections such as human immunodeficiency virus and mycobacterial infections which do not respond well to single-drug therapy, either due to lack of efficacy or rapid emergence of resistance (guidelines for the use of antiretroviral agents in human immunodeficiency virus-infected adults and adolescents are available at http//www.hivatis.org) (10). However, although there are some data on the interaction of antifungal agents against yeasts such as *Cryptococcus neoformans* (21), little is known on the interaction between antifungal agents against filamentous fungi.

In order to determine the potential use of combinations of antifungal agents to treat infections due to *A. fumigatus* we set out to study the in vitro interactions between AM, IT, and terbinafine (TB) using a checkerboard method. Furthermore,

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we investigated whether in vitro resistance to IT could be overcome by combining the drug with other antifungal agents.

One of the problems that arise in such studies is how to describe or define drug interaction. Although the fractional inhibitory concentration (FIC) index is most frequently used (11), there are some important disadvantages. The first one is that there is no clear definition of MICs for filamentous fungi. Growth is inhibited increasingly over a range of twofold dilutions instead of growth versus no growth, as is the case for most antibacterials. Because of this it is not always clear at which MIC endpoint the combination should be read and, thus, how to determine the FIC index. This problem arises especially when a combination of antifungal agents is used with agents that have different MIC endpoints. For example, MIC-0 (100% growth inhibition) is taken as an endpoint for AM, and MIC-2 (≥50% growth inhibition) is taken as an endpoint for IT (15). When these two drugs are combined the question arises which MIC endpoint should be taken for determining the interaction: MIC-0 or MIC-2. A second problem is the definition of the interpretation of the FIC index itself. Various definitions have been described in literature (3, 11, 16, 22, 23). Finally, due to heterogeneous growth of filamentous fungi visual reading is recommended, which is less accurate than spectrophotometric reading.

To overcome these problems, we used a newly developed method based on MTT [3-(4,5-dimethyl-2-thiazyl)2,5-diphenyl-2H-tetrazolium bromide] reduction, which we described recently (13). This method permits the reading of the degree of antifungal activity by a spectrophotometer, and thus quantitative data are obtained. The use of these data then allows the use of response surface modeling, such as that described by Greco et al. (8) or others. By fitting a model to the whole response surface, instead of using only MIC endpoints, an objective criterion for the interaction is obtained, which can be further characterized by statistical analysis and confidence intervals. This approach has been used successfully to determine the interaction of antiviral drugs (5, 18). A new program was developed to allow us to do this analysis with *Aspergillus* spp. The purpose of this study, therefore, was to introduce the response surface model, as described by Greco et al., for determining the interaction of antifungal agents and to compare this model with the FIC index.

(The results presented in this work were partly presented at the 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, 2000, Toronto, Canada.)

MATERIALS AND METHODS

Test isolates. Fifteen clinical isolates of *A. fumigatus* were tested. These included 10 IT-susceptible (IT-S) isolates (AZN 5161, AZN 7151, AZN 7319, AZN 7820, AZN 8248, AZN v02-31, AZN v02-32, AZN v02-33, AZN v02-40, and AZN v02-41) and five IT-resistant (IT-R) isolates (AZN 58, AZN 59, AZN 5241, AZN 5242, and AZG 7). The AZN numbered strains were obtained from the private collection of the Department of Medical Microbiology, University Medical Center Nijmegen, and strain AZG 7 was obtained from the University Hospital Groningen, Groningen, The Netherlands (P. E. Verweij, A. J. M. M. Rijs, J. P. Donnelly, and J. F. G. M. Meis, Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother., abstr. J-33, 1998). The isolates were grown on potato flake agar at 35°C for 5 to 7 days, and conidia were collected. All isolates were tested in triplicate. *Candida parapsilosis* (ATCC 22019) and *Candida krusei* (ATCC 6258) were used for quality control in all experiments.

Antifungal agents. AM (Bristol-Myers Squibb, Woerden, The Netherlands), IT (Janssen Pharmaceutica B. V., Tilburg, The Netherlands), and TB (Novartis B. V., Uden, The Netherlands) were obtained as powders and dissolved in dimethyl sulfoxide to make a stock solution that can be held for 6 months at −70°C.

Serial twofold dilutions of each antifungal agent were prepared following NCCLS guidelines (15). Final dilutions were made in RPMI 1640 medium (with L-glutamine, without bicarbonate) (GIBCO BRL, Life Technologies, Woerden, The Netherlands) buffered to pH 7.0 with morpholinepropanesulfonic acid (MOPS) (0.165 mol/liter; Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

Susceptibility testing. MICs were determined by a modified broth microdilution method that employs the dye MTT (13). The final concentrations of the antifungal agents ranged from 0.016 to 16 μg/ml for all three drugs. Aliquots of 100 μl of the drug at a concentration of two times the targeted final concentration were dispensed in the wells of flat-bottom 96-well microtiter plates (Costar, Corning, N.Y.).

Conidia suspensions were prepared spectrophotometrically (15) and were further diluted in RPMI 1640 medium containing MTT (0.2 mg/ml; Sigma Chemical, St. Louis, Mo.). In order to obtain a final inoculum concentration of 0.4×10^4 to 5×10^4 CFU/ml and a final MTT concentration of 0.1 mg/ml (at this concentration MTT has no antifungal activity [13]), 100 μl of the inoculum was added to the wells. The microtiter plates were incubated at 35°C for 48 h.

After 48 h the content of each well was removed, and 200 μl of isopropanol containing 5% HCl (1 N) was added to extract the dye. After 30 min of incubation at room temperature and gentle agitation, the optical density at 540 nm (OD_{540}) was measured with a microplate reader (Anthos htIII; Anthos Labtec Instruments, Salzburg, Austria). The OD of the blank wells was subtracted from the OD of the inoculated wells.

The percentage of MTT conversion to its formazan derivate for each well was calculated by comparing the OD_{540} of the wells with that of the drug-free control based on the following equation: (OD_{540} of wells that contained the drug/OD_{540} of the drug-free well) × 100%.

The MIC of AM was defined as the lowest concentration that inhibited growth by 95% or more compared with that of the drug-free well (MIC-0). The MICs of IT and TB were defined as the lowest concentration that inhibited growth by 75% or more compared with that of the drug-free well (MIC-1) (13a).

Interaction of drugs in vitro. Drug interactions were assessed by a modified checkerboard broth microdilution method that employs the dye MTT. The final concentrations of the antifungal agents ranged from 0.016 to 16 μg/ml for AM and IT and from 0.031 to 32 μg/ml for TB. Aliquots of 50 μl of each drug at a concentration four times the targeted final concentration were dispensed in the wells.

Conidia suspensions were prepared, and the conversion of MTT was measured as described above.

The following MIC endpoints were used for all three drugs: MIC-0 (≥95% growth inhibition in comparison with the drug-free well), MIC-1 (≥75% growth inhibition), and MIC-2 (≥50% growth inhibition).

Definitions. Drug interaction was determined by the FIC index and by the interaction coefficient alpha (ICα) as described by Greco et al. (8).

The FIC index was defined as follows (11): (MIC of drug A, tested in combination)/(MIC of drug A, tested alone) + (MIC of drug B, tested in combination)/(MIC of drug B, tested alone). The interaction was defined as synergistic if the FIC index was <1, additive if the FIC index was = 1 and antagonistic if the FIC index was >1 (3).

Response surface modeling following Greco et al. (8) is described by the following formula:

$$1 = \frac{D_1}{\mathrm{IC}_{50,1}\left(\frac{E}{\mathrm{Econ} - E}\right)^{\frac{1}{m_1}}} + \frac{D_2}{\mathrm{IC}_{50,2}\left(\frac{E}{\mathrm{Econ} - E}\right)^{\frac{1}{m_2}}} + \alpha * I$$

where

$$I = \left[\frac{D_1 D_2}{\mathrm{IC}_{50,1}\mathrm{IC}_{50,2}\left(\frac{E}{\mathrm{Econ} - E}\right)^{\left(\frac{1}{2m_1} + \frac{1}{2m_2}\right)}}\right]$$

D_1 and D_2 are the concentrations of drug 1 and drug 2, $IC_{50,1}$ and $IC_{50,2}$ are the concentrations of drug 1 and drug 2 resulting in 50% inhibition, E is the measured response, Econ is the control response, m_1 and m_2 are the slope parameters for drug 1 and 2 in a constant ratio, and α is the synergism-antagonism interaction parameter (ICα).

A computer program (ModLab, Medimatics, Maastricht, The Netherlands) was developed to fit this model to the data. The program also determined the

TABLE 1. In vitro interaction between AM, IT, and TB determined by the FIC index for IT-S and IT-R strains[a]

Drug combination	FIC (interaction[b])			
	IT-S (n = 10)		IT-R (n = 5)	
	Mean	Range	Mean	Range
AM-IT	2.077 (ANT)	1.500–2.500	1.700 (ANT)	1.500–2.500
AM-TB	1.654 (ANT)	1.000–2.500	1.392 (ANT)	1.187–1.750
IT-TB	0.656 (SYN)	0.458–0.854	0.412 (SYN)	0.276–0.834

[a] The following MIC endpoints are used: MIC-0 for AM, MIC-1 for IT and TB, and endpoints of ≥95% for the AM-IT and AM-TB combinations and ≥75% for the IT-TB combination.
[b] Interactions: SYN, synergism (FIC < 1); ANT, antagonism (FIC > 1).

95% confidence interval for each parameter. Validation of obtained parameters was done using the program Syner, kindly provided to us by G. L. Drusano.

If the estimate of ICα is zero, the combination is additive. If it is positive, the interaction is synergistic. If it is negative, the interaction is antagonistic. The estimate of ICα has an associated 95% confidence interval. If the confidence interval does not overlap zero, this provides the statistical significance for the estimate of the interaction. That is, if the 95% confidence interval crosses zero, the interaction is additive. If it does not and ICα is positive, the interaction is significantly synergistic. If it does not and ICα is negative, the interaction is significantly antagonistic (5).

Reproducibility of the FIC index and ICα. We have calculated the reproducibility of both the FIC index and the ICα for all three combinations. For each combination the number of strains which had the same interaction for all triplicates was summed (n, with n_{max} = 15). The reproducibility was defined as $(n/n_{max}) \times 100\%$.

Correlation between FIC index and ICα. The correlation between the mean FIC indices, for several MIC endpoints, and the mean ICα was determined by Spearman's correlation coefficient r; a P of 0.05 was considered significant (two sided).

RESULTS

All *A. fumigatus* isolates grew well after 48 h of incubation at 35°C. In each batch of broth microdilution tests, the MICs of the quality control strains were within the reference ranges.

MIC data. The MICs of AM ranged from 0.5 to 2 μg/ml, with a MIC at which 50% of the strains tested were inhibited (MIC_{50}) of 1 μg/ml. The MICs of TB ranged from 2 to >16 μg/ml, with a MIC_{50} of 4 μg/ml. For the IT-S strains the MICs of IT ranged from 0.25 to 2 μg/ml, with a MIC_{50} of 0.25 μg/ml. For all five IT-R strains the MIC of IT was >16 μg/ml.

FIC index. Table 1 summarizes the in vitro interactions determined by the FIC index of the 15 *A. fumigatus* isolates as determined by the MTT method for the AM-IT, AM-TB, and IT-TB combinations. The following MIC endpoints are used: MIC-0 for AM, MIC-1 for IT and TB, and endpoints of ≥95% growth inhibition for the AM-IT and AM-TB combinations and ≥75% growth inhibition for the IT-TB combinations. For both the IT-S and the IT-R strains the AM-IT and AM-TB combinations were antagonistic, although the antagonism was less pronounced for AM-TB. The IT-TB combination was synergistic for all 15 strains. For all five IT-R strains the MIC of IT was lowered from >16 to ≤4 μg/ml when combined with TB. Three of the five strains even had a MIC that was ≤1 μg/ml.

Table 2 shows the mean FIC indices of the IT-S and the IT-R strains for the three combinations determined at various MIC endpoints. Depending on the MIC endpoint used, the FIC index ranged from 1.016 to 2.077 for AM-IT, from 0.544 to 1.767 for AM-TB, and from 0.656 to 0.740 for IT-TB for the IT-S strains. For the IT-R strains the FIC index ranged from 0.308 to 1.767 for AM-IT, from 0.512 to 1.646 for AM-TB, and from 0.403 to 0.497 for IT-TB. The results indicate that the degree of interaction is determined not only by the agents themselves but also by the choice of the endpoint.

Response surface model. Figure 1 shows an example of the interaction surface of the AM-IT combination for one of the IT-S strains. Model fits to the data using the Greco et al. model were performed for each strain and drug combination. Table 3 shows an example of the results found for the IT-TB combination for all the strains for one set of replicates. The results of the replicates of each experiment (each experiment was per-

TABLE 2. Mean FIC indices of various drug combinations for IT-S and IT-R strains at various MIC endpoints

MIC endpoint[a]	FIC for:					
	IT-S (n = 10)			IT-R (n = 5)		
	AM-IT	AM-TB	IT-TB	AM-IT	AM-TB	IT-TB
1	1.800	0.934	0.713	1.551	0.815	0.497
2	2.077	1.654	0.656	1.700	1.392	0.412
3	1.992	1.767	0.656	1.767	1.646	0.412
4	1.510	0.861	0.740	0.840	0.708	0.403
5	1.016	0.544	0.740	0.308	0.512	0.403
6	1.894	1.187	0.656	1.304	0.875	0.412
7	1.519	1.051	0.740	0.975	0.809	0.403

[a] Endpoints: 1, MIC-0 for AM, IT, and TB and an endpoint of ≥95% for all three combinations; 2, MIC-0 for AM, MIC-1 for IT and TB, and endpoints of ≥95% for the AM-IT and AM-TB combinations and ≥75% for the IT-TB combination; 3, MIC-0 for AM, MIC-1 for IT and TB, and an endpoint of ≥75% for all three combinations; 4, MIC-0 for AM, MIC-2 for IT and TB, and endpoints of ≥95% for the AM-IT and AM-TB combinations and ≥50% for the IT-TB combination; 5, MIC-0 for AM, MIC-2 for IT and TB, and an endpoint of ≥50% for all three combinations; 6, MIC-1 for AM, IT, and TB and an endpoint of ≥75% for all three combinations; 7, MIC-2 for AM, IT, and TB and an endpoint of ≥50% for all three combinations.

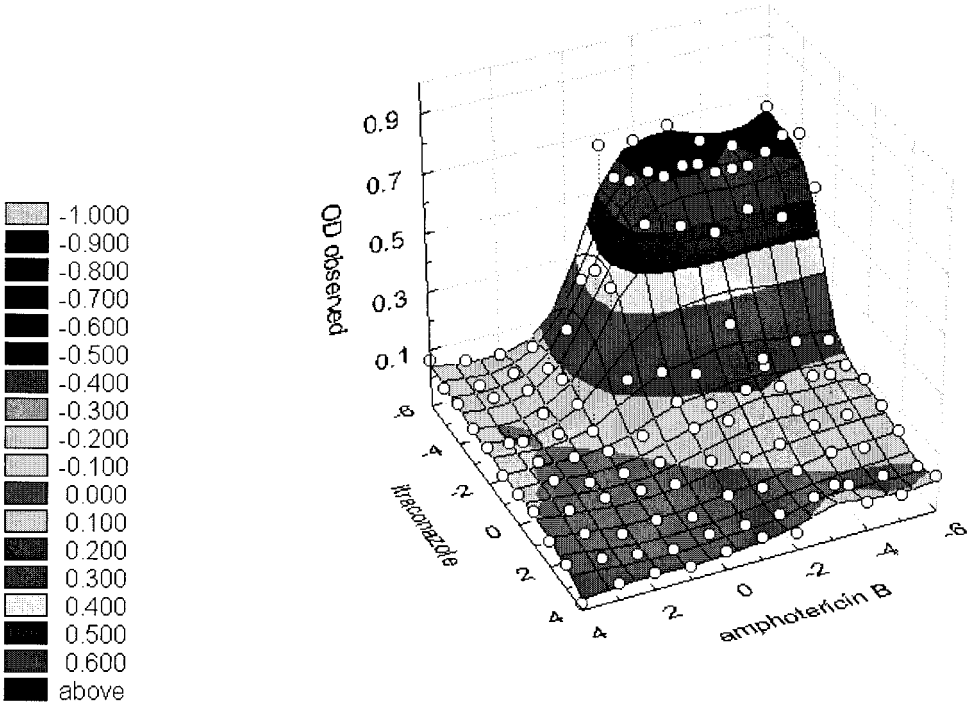

FIG. 1. Interaction surface of AM-IT for an *A. fumigatus* strain.

formed in triplicate) yielded similar conclusions. However, for some strains analyses were not consistent, in that one of the replicates yielded an ICα opposite to the other replicates. In most cases, these values were very close to zero. Table 4 summarizes the in vitro interaction coefficients found for all experiments. The general conclusions from this table are that for both the IT-S and the IT-R strains the AM-IT and AM-TB combinations were antagonistic. The IT-TB combination was synergistic for both IT-S and IT-R strains.

TABLE 3. ICαs for the IT-TB combination for all strains

Strain	ICα	95% CI[c]	Interaction[b]
AZN 5161	0.414	0.175 to 0.977	SYN
AZN 7151	0.134	0.056 to 0.322	SYN
AZN 7319	0.213	0.071 to 0.637	SYN
AZN 7820	−0.046	−0.002 to −0.999	ANT
AZN 8248	0.484	0.224 to 1.045	SYN
AZN v02–31	0.234	0.107 to 0.511	SYN
AZN v02–32	0.694	0.424 to 1.138	SYN
AZN v02–33	ND[a]	ND	ND
AZN v02–40	ND	ND	ND
AZN v02–41	0.346	0.159 to 0.756	SYN
AZN 58	ND	ND	ND
AZN 59	14.70	7.794 to 27.73	SYN
AZN 5241	0.592	0.244 to 1.438	SYN
AZN 5242	8.219	4.822 to 14.01	SYN
AZG 7	1.665	1.101 to 2.518	SYN

[a] ND, no model fits obtained.
[b] Synergism (SYN) was defined as an ICα of >0, and antagonism (ANT) was defined as an ICα of <0 and a 95% confidence interval that did not include 0. If the 95% confidence interval included 0 the interaction was considered to be additive. Conclusions regarding antagonism, additivity, or synergism are dependent on 0 falling within the 95% confidence interval.
[c] 95% CI, 95% confidence interval.

Reproducibility of FIC index and ICα. Table 5 shows the reproducibility of both the FIC index and the ICα for each combination. The table shows that the ICα has a reproducibility of 93% for all three combinations, while the reproducibility of the FIC index varies between 53 and 100%.

Correlation between FIC index and ICα. Correlation between the mean FIC indices and the mean ICαs for all the combinations together ($n = 45$), was determined for the different MIC endpoints that are mentioned in Table 2. The highest significant correlation was found for the following MIC endpoints: MIC-0 for AM, MIC-1 for IT and TB, and endpoints of ≥95% growth inhibition for the AM-IT and AM-TB combinations and ≥75% growth inhibition for the IT-TB combination. For these MIC endpoints Spearman's correlation coefficient r was −0.7187 ($P < 0.0001$).

Figure 2 shows the relation between the mean FIC indices and the mean ICαs for the 15 strains for all the three combinations together.

DISCUSSION

In this study we show that the MTT method can be used to determine the interaction between antifungal drugs against filamentous fungi by reading the degree of antifungal activity with a spectrophotometer. Furthermore, we showed that by using the MTT-based method response surface modeling can be applied to study drug-drug interaction in *Aspergillus* and is more reproducible than using FIC indices.

The FIC index is the most frequently used method to determine the interaction between antifungal drugs. However, this method has some important disadvantages. First of all it is not clear at which MIC endpoint the combination should be read.

TABLE 4. ICαs for combinations of AM, IT, and TB determined by the Greco et al. model for IT-S and IT-R strains

Drug combination	ICα (interaction[a]) for:			
	IT-S (n = 10)		IT-R (n = 5)	
	Mean	Range	Mean	Range
AM-IT	−0.054 (ANT)	−0.020– −0.088	−0.172 (ANT)	−0.061– −0.245
AM-TB	−0.076 (ANT)	−0.015– −0.118	−0.063 (ANT)	−0.035– −0.112
IT-TB	0.574 (SYN)	0.072–1.755	4.349 (SYN)	0.697–7.546

[a] Interactions: SYN, synergism (ICα > 0); ANT, antagonism (ICα < 0).

This problem especially arises when a combination of drugs with different MIC endpoints is used, for example the combination of AM and IT. Reading the MIC of the drug interaction using different endpoints resulted in different FIC indices, leading to different interpretations of the interaction, as shown in Table 2. Indeed, any conclusion based on the FIC indices is more dependent on the choice of endpoint, which is more or less arbitrary, than on the results of the experiments themselves for some drug combinations. The values for the AM-IT combination for instance, varies between 0.308 and 2.007, respectively, depending on the chosen endpoints. Conflicting reports in the literature regarding synergism and antagonism between two drugs may be partly due to this. Sometimes different endpoints are used for the same combination or the chosen endpoints are not mentioned at all (1, 2, 4, 20, 22). The use of a model fit to the whole data surface without the necessity of arbitrarily choosing an endpoint is therefore preferred.

A second disadvantage of the FIC method is that there are different definitions described for the interpretation of the FIC index and it is not clear which interpretation should be used (3, 11, 16, 22, 23). In another study in which we looked at the interaction between flucytosine and fluconazole against various strains of *Candida* and *Cryptococcus*, the conclusions based on four different criteria of interpretation were compared (J. W. Mouton, Abstr. 10th Eur. Cong. Clin. Microbiol. Infect. Dis., abstr. TuS7, 2000). Depending on the definition, synergism was found between 0 and 20% of strains (n = 35), while antagonism varied as much as from 29 to 77% of strains. Not only the definitions but also the lack of a statistical criterion to define these interactions contribute to these varying results. The use of a model fit at least allows an objective statistical criterion.

A final shortcoming of the use of FIC indices in determining interaction of drugs against filamentous fungi is that heterogeneous growth, which is characteristic for filamentous fungi, prohibits objective reading from the wells, resulting in the need for visual readings (15).

Fitting of a model to the whole data surface not only allows the optimal use of information in the data but also allows the determination of error estimates of the interaction coefficient, thereby indicating whether the interaction is significant or not. Since the effect of drug interaction may vary for strains, this is an important feature. Error estimates of FIC indices are generally not determined, and if so can only be determined by performing multiple replicate experiments.

The bounds of the 95% confidence intervals of the estimate of the interaction coefficient thus can be used to determine whether interaction is present, depending on whether the value of 0, which indicates no interaction is present, falls between that interval.

Not only does the interaction coefficient indicate whether there is significance or not, but the value found also gives an indication of the degree of interaction. This is not unlike the value of an FIC index, although on a different order, and in the present study we compared the values of the interaction coefficients found with the FIC indices. Although the problem arises that the absolute value of the FIC index differs in relation to the endpoint chosen as discussed above, there is a degree of relativity. For instance, if the efficacy of the combination against two strains is compared and the FIC index for one strain is lower than that for the other using one set of endpoints, it will usually be lower as well using another set of endpoints. Thus, when the results of the FIC indices obtained by using different endpoints were combined, there was a rea-

TABLE 5. Reproducibility of both FIC index and ICα for each combination

Drug combination	% Reproducibility (interaction[s])[a]	
	FIC index	ICα
AM-IT	100 (15 ANT)	93 (14 ANT)
AM-TB	53 (7 ANT, 1 ADD)	93 (14 ANT)
IT-TB	67 (10 SYN)	93 (14 SYN)

[a] Values in parentheses indicate the number of strains with the same interaction for the three replicates out of all strains tested (n = 15). Abbreviations for interactions: ANT, antagonism; ADD, addition; SYN, synergism.

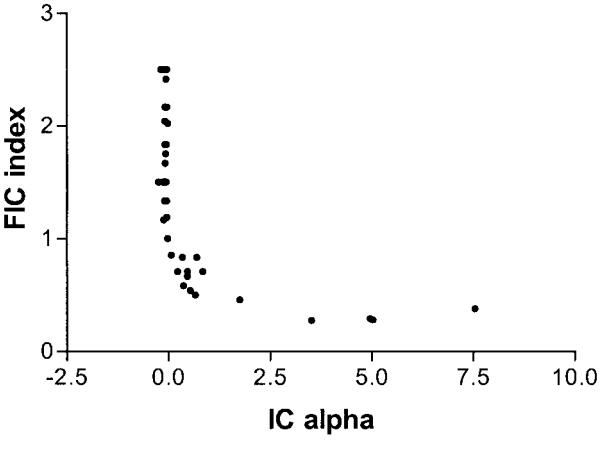

FIG. 2. Relation between the mean FIC indices and the mean ICαs of the Greco et al. model.

sonably good correlation between the various approaches. Also, the FIC indices correlated well with the interactions coefficients found by the Greco et al. model, depending on the MIC endpoint. There was even concordance between FIC index interpretation and the interpretation using the interaction coefficient (Tables 1 and 4).

To be able to use quantitative continuous data, we used a modified MTT method (13). This method is based on the reduction of dye MTT to formazan by viable fungi and has been shown to correspond with results obtained by the NCCLS method when determining MICs, but without the disadvantage of a possible observer bias. Our results show that this method is suitable for interaction studies as well.

Of the combinations tested, the most potent in vitro combination was the combination of IT with TB. This combination was synergistic for both IT-S and-R strains, although synergism seemed to be slightly more pronounced for the IT-R strains, and concurs with earlier studies (20; N. S. Ryder and I. Leitner, Abstr. 36th Intersci. Conf. Antimicrob. Agents Chemother., abstr. E54, 1996). For all five IT-R strains the apparent in vitro resistance was overcome by combining IT with TB. Previous studies have already suggested that azole-resistant fungi can be treated with TB in combination with azoles (7; A. W. Fothergill, I. Leitner, J. G. Meingassner, N. S. Ryder, and M. G. Rinaldi, Abstr. 36th Intersci. Conf. Antimicrob. Agents Chemother., abstr. E53, 1996; L. Rodero, R. Vitale, F. Hochenfellner, C. Canteros, and G. Davel, Abstr. 36th Intersci. Conf. Antimicrob. Agents Chemother., abstr. E56, 1996).

The synergism between these two agents can be explained on the basis of their mode of action. IT and TB block different steps in the ergosterol biosynthesis. IT inhibits through its action on the cytochrome P-450-dependent enzyme lanosterol 14α-demethylase (6). TB on the other hand inhibits the action of squalene epoxidase, a crucial enzyme in the formation of ergosterol (6). Thus, this is another example of synergism between two drugs that block different steps of the same pathway, similar to the combination of trimethoprim with sulfonamides (19).

Both IT and TB showed in vitro antagonism in combination with AM. In previous in vitro studies the combination of AM and IT showed different interactions (4, 12). These differences may have been the result of the use of different methods and different strains and, more probably, the use of the FIC index.

We conclude that the use of a colorimetric method and consequent analysis by using a model fit to the response surface is a useful approach to determine the interaction between drugs against filamentous fungi and has considerable advantages over conventional methods. The validation of this method shall be done in an experimental model of *Aspergillus* infection.

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